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L4: Entry 3 of 5 File: USPT Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5767369 A
TITLE: DNA sequences encoding SAR8.2 proteins and uses thereof

INZZ:
Goodman; Robert M.

BSPR:
Advances in recombinant DNA technology coupled with advances in plant transformation and regeneration technology have made it possible to introduce new genetic material into plant cells, plants or plant tissue, thus introducing new traits, e.g., phenotypes, that enhance the value of the plant or plant tissue. Recent demonstrations of genetically engineered plants resistant to pathogens (EP-A 240 332 and EP-A 223 452) or insects (Vaeck, M. et al., Nature 328:33 (1987)) and the production of herbicide tolerant plants (DeBlock, M. et al., EMBO J. 6:2513 (1987)) highlight the potential for crop improvement. The target crops can range from trees and shrubs to ornamental flowers and field crops. Indeed, it is clear that the "crop" can also be a culture of plant tissue grown in a bioreactor as a source for some natural product.

BSPR:
Plant cell cultures can be established from an impressive array of plant species and may be propagated in a bioreactor. Typical plant species include most of those that produce secondary products of commercial interest. It has been clearly demonstrated in a number of agriculturally important crop plants that stable genetic variants arising from the tissue culture of plant somatic cells (somaclonal variation) can be induced and selected. Numerous advantages flow from plant tissue culture production of secondary compounds. These include (1) the possibility of increased purity of the resultant product, (2) the conversion of inexpensive precursors into expensive end products by biotransformation, and (3) the potential for feeding substrate analogs to the culture to create novel compounds.

BSPR:
Whether the target of genetic engineering of plants is a field crop, ornamental shrub, flower, tree or a tissue culture for use in a bioreactor, a principal advantage to be realized is the control of expression of the chimeric gene so that it is expressed only at the appropriate time and to the appropriate extent, and in some situations in particular parts of the plant. For example, in order to achieve a desirable phenotyye the chimeric gene may need to be expressed at levels of 1% of the

total protein or higher. This may well be the case for fungal resistance due to chimeric chitinase expression or insect resistance due to increased proteinase inhibitor expression. In these cases the energy expended to produce high levels of the foreign protein may result in a detriment to the plant whereas, if the gene were expressed only when desired, for instance when a fungal or insect infestation is imminent, the drain on energy, and therefore yield, could be reduced.

BSPR:
For tissue in culture or in a bioreactor the untimely production of a secondary product could lead to a decrease in the growth rate of the culture resulting in a decrease in the yield of the product. Therefore, it would be advantageous to allow the culture to grow without expressing the secondary product and then induce the chimeric gene at an appropriate time to allow for an optimized expression of the desired product.

BSPR:
In view of considerations like these, as well as others, it is clear that control of the time, extent and/or site of expression of the chimeric gene in plants or plant tissues would be highly desirable. Control that could be exercised easily in a field, a greenhouse or a bioreactor would be of particular commercial value.

BSPR:
The first aspect of the invention further embraces several uses of the chemically regulatable DNA sequences: (a) regulation of chimeric genes in cells propagated in a bioreactor, (b) an assay for chemical regulators, (c) developmental regulation of the plant, (d) regulation of plant sterility and (e) regulation of chimeric and/or heterologous gene expression in a transformed plant. Other uses and advantages will be apparent from the following detailed description of the invention.

DEPR:
The chimeric genes described above embrace a variety of possible constructions. A chemically regulatable non-coding sequence can be associated with a gene controlling flowering or fruit ripening; a gene effecting tolerance or resistance to herbicides or to many types of pests, for example fungi, viruses, bacteria, insects, nematodes, or arachnids; a gene controlling production of enzymes or secondary metabolites; male or female sterility; dwarfness; flavor; nutritional qualities; and the like. Using the present invention such traits can be enhanced by the farmer and gardener, which is no longer dependent on natural factors alone. A phenotypic trait of particular interest for control is the production of metabolites in tissue culture or a bioreactor.

DEPR:
The cells transformed may originate from monocotyledenous or dicotyledonous plants and may contain one or more of the chemically regulatable chimeric genes of this invention. Thus, genes which, for example, code for resistance or tolerance to herbicides and a variety of insect, viral, bacterial, fungal and other pests, for sterility, for size, for flowering and fruit

ripening, are introduced in the plant tissue, and these cells or protoplasts ultimately regenerated into plants in which these traits can be controlled by manipulations with a chemical regulator. Alternatively cells can be propagated in tissue culture or in a bioreactor to produce enzymes or secondary matabolites. If an enzyme assay is desired, the coding section of the chimeric gene may, for example, comprise a LUX, CAT, NPT, NOS, OCS, GUS, AHAS or BT gene, as identified previously. Such chimeric genes containing a chemically inducible sequence from a PR gene are a preferred embodiment of the invention because of the ease of application of the regulator and the ease of detection of the enzyme product.

DEPR:
Effecting the control may be accomplished simply by applying the chemical regulator to the plant tissue, or to the plant or part of the plant in such a manner and in such an amount to regulate the chimeric gene(s) whose expression in plant cells, plant tissues or plants is desired. For example, if the trait to be expressed is preferably expressed only in the leaves, then spraying or dusting the leaves at a time which optimizes that expression in the leaves, and before any migration to other parts of the plant, may accomplish that objective easily and efficiently. Alternatively uniform expression throughout that part of the plant above ground may result from application to the entire plant (i.e., stem and both sides of the leaves). If expression in the roots is desired, application to the seeds or the soil around the seeds or roots is a possible method of regulation. Expression in a bioreactor is accomplished quite easily, for example, by applying the chemical regulator to the medium contacting the cells.

DEPR:
In certain situations, it would be desirable to regulate the expression of various heterologous genes (transgenes) in transgenic plants. For example, the effectiveness of disease-or insect resistance in transgenic plants transformed with genes encoding disease- or insect-resistant proteins, respectively, could be enhanced if the timing of the expression could be controlled. See, e.g., Uknes, Plant Cell, 4:645-656 (1992); Ward et al., Plant Cell 3:1085-1094 (1991); Gould, Bioscience 38:26-33 (1988); and Gould, TIBTECH 6:S15-S18 (1988). Also, the chemical regulation o developmental processes such as homeosis, germination, tillering, sprouting, flowering, anthesis, fruit ripening, and abscission offers several advantages such as the facilitated production of hybrid seed, greater reduction of crop loss, and more generally, control of the growth and development of the plant by the farmer. Thus, the present invention applies equally to transgenic plants containing heterologous genes, e.g., disease resistance genes including PR and SAR genes, insect resistance genes such as Bt genes, and genes involved in developmental processes such as those described above. It also includes genes encoding industrial or pharmaceutical biomaterials such as plastics and precursors thereof, perfumes, additives, enzymes and other proteins, and pharmaceutical, wherein the plant effectively would be used as a bioreactor, e.g., the two genes encoding production of polyhydroxybutyrate, a thermoplastic

(Poirer et al., Science 256:520-523 (1992). To practice this embodiment of the present invention, the heterologous gene of interest should be fused to a promoter capable of being regulated by the exogenous chemical regulator (eg. containing a chemically regulatable DNA sequence) and for which activity, the signal is not required exclusively. In other words, the promoter can be regulatable by the signal, provided that it can be regulated by a chemical regulator in the absence of a functional, endogenous signal. Examples include the PR-1a promoter such as those disclosed herein and in Williams et al., Bio/Technology 10:540-543 (1992); Uknes et al., The Plant Cell 5:159-169 (1993); and Van de Rhee et al., Plant Cell 2:357-366 (1990), and other promoters isolated from chemically regulated plant genes such as those described herein and in Payne et al., Plant Mol. Biol. 11:89-94 (1988).

DEPR:
The chemical regulators may be applied in pure form, in solution or suspension, as powders or dusts, or in other conventional formulations used agriculturally or in bioreactor processes. Such formulations may include solid or liquid carriers, that is, materials with which the regulator is combined to facilitate application to the plant, tissue, cell or tissue culture, or the like, or to improve storage, handling or transport properties. Examples of suitable carriers include silicates, clays, carbon, sulfur, resins, alcohols, ketones, aromatic hydrocarbons, and the like. If formulated as a conventional wettable powder or aqueous emulsion, the regulator formulation may include one or more conventional surfactants, either ionic or non-ionic, such as wetting, emulsifying or dispersing agents.

DEPR:
As a liquid formulation the regulator may be applied as a spray to plant leaves, stems or branches, to seeds before planting or to the soil or other growing medium supporting the plant. Regulators can also be used in bioreactor systems, regulation being achieved by a single addition of regulator formulation to the reaction medium or by gradual addition over a predetermined period of time.